Mycofloral Succession and Viability Losses in Pigeon pea Seed in Puerto Rico[1]

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ABSTRACT

Using the cellulose pad and potato dextrose agar (PDA) assays, twenty three fungi were found on pigeonpea seed in Puerto Rico. In the PDA assay, *Botryodiplodia theobromae* was the most common (29%). On cellulose pads, the same fungus was not very common (7%). PDA assay favored increased detection of *Alternaria tenuissima*, *Phomopsis* sp., and total fungi compared with cellulose pads. Cellulose pads favored detection of *Cladosporium* sp. In both assays, incidences of *B. theobromae*, *Fusarium* spp., and total fungi were negatively correlated with seed germination. Measurements for seedlot germination were highly correlated ($r = 0.77^{**}$) between the two assays.

The influences of seed type and delayed harvest on pigeonpea seed viability were studied. Pigeonpeas "2B-Bushy," with large tan seeds, showed reduced seed viability and higher incidence of *B. theobromae* and *Fusarium* spp. compared with segregants of the same cultivar with small flattened hard red seed. A 3-week delayed harvest drastically increased incidence of *B. theobromae*, *Fusarium* spp., and total fungi, and reduced germination in "Kaki" pigeonpeas from Santa Isabel.

Pigeonpea seed viability and mycoflora were followed before and after farm storage. Incidences of *Fusarium* spp., *Phomopsis* sp., *Cladosporium* sp., and *B. theobromae* after storage were 15, 26, 30, and 38% of their respective prestorage values. Incidence of species of *Penicillium*, *Rhizopus*, and *Aspergillus* increased during storage. Although less than 3% *Aspergillus* spp. was detected in the cellulose pad assay at 27° C, 28 to 92% was detected when seed were incubated at 35° C using pigeonpea seed extract in 2% agar. With the *Aspergillus* selective assay, seed viability losses during storage were highly correlated with incidence of *Aspergillus* spp. ($r = 0.96^{**}$). *Aspergillus* incidence in the cellulose pad assay was not associated with storage losses in seed germination ($r = 0.18$ NS).

Emergence and fungal colonization of pigeonpea seed were dependent on soil moisture. Pigeonpea seed did not emerge at either 25 or 100% soil moisture holding capacity (SMHC). Optimum emergence was found at 50% SMHC. Pythiaceous fungi were predominent on seed at and above 75% SMHC, whereas *Aspergillus* spp. predominated at 50% SMHC or less. Mixed populations of the two were visible at 50 and 75% SMHC.

INTRODUCTION

Pigeon pea (*Cajanus cajan* (L.) Millsp.) is a short-lived perennial bush legume which is used for food and feed in tropical areas (6, 10). Pigeon pea seed contains from 16–30% crude protein on a dry weight basis (6, 10). When consumed with cereals or root crops, pigeon peas are helpful in balancing essential amino acid content, thus increasing protein utilization. For this reason pigeon peas are important in low cost staple diets. Although nearly 90% of all pigeonpea production is centered on the

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Indian subcontinent, significant production of pigeon peas is found in most tropical countries. In India, pigeon peas are consumed as dry split peas (dhal), whereas, in the West Indies peas are harvested as immature peas and eaten either fresh or canned. In eastern Africa, pigeon peas are most popular with certain tribes. Most of the production of pigeon peas is on small holdings destined for home consumption or for the local market. Because pigeon peas often do not appear in large commercial markets, their economic value is difficult to assess and is often underestimated. In Puerto Rico, about 6,000 ha are devoted to the commercial production of pigeon pea. Farm value exceeds $6 million annually and supports in part the activity of two local canneries (5).

Pigeon peas are often grown on marginal land because of their tolerance to low fertility and periodic drought (6, 10). Supplemental supplies of major nutrients have not been shown to increase either harvest yield or quality, even on low fertility soils in Puerto Rico (9). Because of the crop characteristics, pigeon peas have become a priority crop at the International Crops Institute for the Semi-arid Tropics (ICRISAT).

According to a survey of world literature, there are 20 fungi, 2 bacteria, and 6 viruses which are known causes of pigeon pea diseases (2). Pigeon peas are often cited for their disease resistance compared with that of dry beans. Like most other crops which are important in subsistence agriculture but less so commercially, pigeon peas have received little scientific investigation compared to most major crops of the temperate zone. Spence (13) has stressed the lack of reliable information from the Carribbean Basin on disease losses in grain legumes; he suggested a need for increased disease survey in order to accumulate information needed to program disease control efforts.

Although there are several diseases of pigeon peas reported in Puerto Rico, there is little or no information on their importance in the field or potential to cause field losses. Ellis et al. (3, 4) suggested that poor seed quality could be a limiting factor. Agarwal (1) in India, however, found no marked effect of seedborne or storage fungi on seed viability. Considering the conflict in these reports it was deemed desirable to fully identify the scope and nature of seed quality losses in Puerto Rico under farmer conditions.

MATERIALS AND METHODS

During 1980–1981, three farms in each of four municipalities in which pigeon peas are a major crop were surveyed biweekly for field disease development. At harvest, seed was obtained from two of the three sites in each municipality. Four random seedlots of 50 seeds each were assayed both on cellulose pads and, after surface disinfection, on agar plates. Cellulose pads (Kimpac) were moistened with distilled water and seeds

without treatment were incubated for 10 days at 27° C. In the agar plate assay seeds were surface treated with 0.5% NaOCl (10% Clorox) by being submerged for 4 minutes. Seeds were then dried under aseptic conditions under a laminar flow hood. Seed was then aseptically transferred and incubated for 7 days at 25° C on sterile potato dextrose agar (PDA, BBL). Seeds were counted as germinated when the radicle extended 2 times the length of the cotyledon at 10 days after first incubation and the radicle appeared both intact and viable. Fungi were identified under the stereoscope (40 ×) and confirmed under the light transmission compound microscope (100 to 1,000 ×).

In the course of the disease survey off-type pigeon pea plants were identified in several commercial fields. In a field in Santa Isabel off-type 2B-Bushy was found to have the general morphology (dwarfness, shape, flower color, and habit) of normal 2B-Bushy but to differ in having a smaller pod and small hard red seed. Twenty off-type plants were harvested along with an equal number of normal neighboring plants. From these plants four random seedlots of 50 seed each were assayed on cellulose pads. The majority of off-type seed did not absorb moisture during incubation and were scarified by being placed in a slurry of sterile quartz sand which was shaken at 30 n/m for 30 minutes. We then rinsed the seed in a No. 60 sieve with running tap water. To determine their viability we removed the seeds and reincubated them on cellulose pads.

A delayed harvest experiment was conducted in a commercial field of Kakai pigeon peas in Santa Isabel. Pods were observed through their stages of maturity and into delayed harvest. After harvest maturity, visible colonization by fungi blackened pods in three weeks. Twenty plants having both clean dry pods (harvest maturity) and blackened pods (three week harvest delay) were harvested and pods were separated on the basis of their maturity. From each harvest maturity four 50-seed lots were selected at random and assayed on agar plates as previously described.

To test viability of seeds under farmer storage, we surveyed seedlots from four farmers of the original eight on the basis of their practice of saving their own seed for subsequent planting. Seed was assayed before and after storage with the cellulose pad assay as previously described. Harvest and first replanting corresponded to December 1980 and May 1981. Since viability losses under on farm storage were not associated with field or storage fungi as detected in the cellulose pad assay, an assay to help detect storage fungi was developed. To increase the detection of *Aspergillus* spp. we made the following modifications: water availability was lowered with a high rate of agar in the test medium; incubation temperature was raised to favor Aspergilli but not field fungi; and a tannin-rich extract from pigeon pea seeds was used because tannins are

known to inhibit many fungi and other microorganisms but are utilized by most Aspergilli. Using a ferric chloride acetate based photometric assay of seed extract (7), we found 2 to 3% tannins. We prepared an extract by boiling 50 g of dry pigeon pea seeds in 1 liter distilled water for 30 minutes. We poured the hot solution through a double layer of cheese-cloth and reconstituted it to 1 liter by adding distilled water. We added twenty grams of agar and stirred the mixture before autoclaving for 30 minutes at 15 lb/in^2 and 121° C. In plating, seeds were first surface disinfected with 0.5% NaOCl for 4 minutes and then aseptically transferred to the sterile medium in sterile plastic petri plates (9 cm). Plates were incubated for 7 days at 35° C.

An organic soil from 1-year-old fermented sugarcane filter mud (cachaza) was used to determine the effect of soil moisture on pigeon pea emergence. Soil was air-dried and then we determined the moisture capacity by measuring moisture gain on four dry samples of 100 grams each, which after complete saturation were drained overnight. The test soil was saturated by approximately 67 ml. Using this information, 200-g samples were adjusted to 25, 50, 75, and 100% water saturation (moisture holding capacity (MHC)). Soil samples were housed in 400 ml glass beakers allowing the observation of germination and seed attack by soil- and seed-borne fungi. Five replicates of 10 seed each were used for each moisture holding capacity test. Beakers were incubated at 27° C and 95% RH for 2 weeks after which time emergence and fungal attack were determined. Seeds used in the test were assayed for germination and seed-borne fungi by the PDA plate test as previously described. Germination of the Kaki seedlot was 55%, with 23% *B. theobromae*, 24% *Fusarium* spp., 10% *Phomopsis* sp., 12% *Cladosporium* sp., and 63% total fungi.

RESULTS

ASSAY OF SEEDBORNE FUNGI BEFORE HARVEST

Table 1 shows germination and incidence of seedborne fungi from pigeonpea fields in Puerto Rico. Recovery of *Phomopsis* sp. and *Penicillium* spp. appeared to be influenced by the elevation of the production field. Higher elevation in this study was associated with increased rainfall and lower temperatures. *Phomopsis* sp. and *Penicillium* spp. were not prevalent at coastal sites and their recovery increased with increased elevation. Differences in the recovery of other fungi based on elevation were not evident. Cellulose pad and potato dextrose agar assays varied in their estimation of germination and seedborne fungi. Increased germination and recovery of *Cladosporium* sp. and *Rhizopus* spp. were found on cellulose pads. In the PDA assay, in which seeds were surface disin-

TABLE 1.—*Germination and seedborne fungi of pigeon pea seedlots from various production sites in Puerto Rico*

Site	Mean percentage (%)[1]													
	Germination		*Botryodiplodia*		*Fusarium*		*Phomopsis*		*Cladosporium*		*Alternaria*		Total fungi	
	CP[2]	PDA[2]	CP	PDA	CP	PDA	CP	PDA	CP	PDA	CP	PDA	CP	PDA
Coamo 1	65	50	18	45	16	12	6	13	4	0	0	0	44	80
Coamo 1	84	66	1	22	1	11	4	6	15	4	0	8	27	70
Villalba 1	73	87	0	13	4	10	0	3	24	2	2	13	34	27
Villalba 2	59	54	0	45	15	33	8	12	15	0	0	0	44	82
Santa Isabel 1	78	43	18	52	10	6	0	1	8	2	0	2	40	75
Santa Isabel 2	27	29	12	10	78	85	0	1	0	0	0	3	90	100
Yauco 1	78	65	8	28	11	11	4	16	4	4	0	8	27	71
Yauco 2	92	77	0	17	2	6	0	3	10	3	2	8	16	40
$\bar{x}$[3]	70	59	7	29	17	22	3	7	10	2	1	5	40	68
Sign.[4]		**		**		NS		*		**		**		**

[1] Individual means based on 4 samples of 50 seeds each.

[2] CP = assay using moist cellulose pads and PDA = agar plate assay using potato dextrose agar, both at 27° C.

[3] Composite mean based on 32 seedlots from 8 sites in pigeon pea production areas in Puerto Rico.

[4] Statistical significance (Sign.) based on Fischer's Least Significant Difference (FLSD); * = Stat. Diff. at P = 0.05, ** = Stat. Diff. at P = 0.01, and NS = No Stat. Diff. at P = 0.05.

fected with NaOCl, recovery of *Phomopsis* sp., *B. theobromae*, and *Alternaria tenuissima* were favored. Incidence of *Fusarium* spp. was not statistically different between the two assays; however, *F. semitectum* was the dominant species on cellulose pads and *F. sambucinum* var. *coeruleum* on PDA plates. A high correlation ($r = 0.78^{**}$) was found between the germination values of the two methods. Table 2 shows the associations of fungal parameters with germination (linear correlation coefficients (r).

EFFECT OF SEED TYPE ON VIABILITY AND MYCOFLORA

Pigeonpea seed cv. 2B-Bushy had either large tan (normal) or small hard red seed (off-type). Over 15 times the incidence of seed fungi were found in the normal seed, which are the preferred vegetable type com-

TABLE 2.—*Linear correlation coefficients (r) for germination values with respect to various fungal parameters from two germination assays*

Parameter	Germination Assay[1]	
	Cellulose Pad[2]	Potato Dextrose Agar[3]
Alternaria tenuissima	+0.408 NS	+0.806*[4]
Botryodiplodia theobromae	−0.315 NS	−0.594*
Cladosporium sp.	+0.416 NS	+0.585*
Fusarium spp.	−0.923**[4]	−0.858**
Phomopsis sp.	−0.042 NS	+0.134NS
Total fungi	−0.974**	−0.928**

[1] Based on 32 50-seed samples for each assay.

[2] Cellulose Pad Assay with Kimpac Cellulose incubated at 27°C.

[3] Potato Dextrose Agar Assay using surface-treated seed (4 min 0.5% NaOCl) incubated on BBL PDA at 27°C.

[4] See Table 1.

pared to the hardseeded off-type (table 3). Hard seed did not readily imbibe water but were viable as shown by their germination after scarification.

EFFECT OF HARVEST DELAY

Delayed harvest of Kaki pigeon peas (3 weeks) reduced germination and increased *B. theobromae*, *Fusarium* spp., and total fungi (table 4). *Fusarium* spp. (49%) were dominant after delayed harvest and *B. theobromae* (18%) at normal harvest. Incidence of *B. theobromae* almost doubled (31%); whereas, *Fusarium* spp. increased more than four times.

STORAGE DETERIORATION

Farmer seed collected at the December harvest and planted in May was used for analyzing viability and mycoflora changes. After farmer

storage, seed viability varied from 49 to 92% of the preharvest values. Field fungi, which were prevalent before storage (*B. theobromae*, *Fusarium* spp., *Phomopsis* sp., and *Cladosporium* sp.), were significantly reduced after storage, whereas *Rhizopus*, *Penicillium*, and *Aspergillus* species increased during the same time (table 5).

SELECTIVE ASSAY

Aspergillus spp. were more frequently recovered in a selective assay (table 6). Average recovery of *Aspergillus* spp. on cellulose pads at 27° C

TABLE 3.—*Effect of hardseededness on the germination, viability, and seedborne fungi of 2B-Bushy pigeon pea*

Seed type	100-seed weight (g)	Mean Percentage (%)[1]					
		Germination	Hardseed[2]	Total viable seed	*Botryo-diplodia*	*Fusarium*	Total fungi
Large Tan	14.0	27	0	27	12	78	90
Small Red	8.6	11	89	100	0	0	5
Sign.[3]	**	**	**	**	**	**	**

[1] Means based on 4 seedlots of 50-seed each per type of seed. Seeds were assayed on cellulose pads at 27° C.

[2] Impermeable seeds were scarified and reincubated on cellulose pads to test their viability.

[3] Statistical Significance (Sign.) based on FLSD; ** = stat. diff. at P = 0.01.

TABLE 4.—*Effect of delayed harvest on seed viability and seed fungi of Kaki pigeon peas grown at Santa Isabel, Puerto Rico*

Maturity[2]	Mean Percentage[1]			
	Germination	*Botryodiplodia*	*Fusarium* spp.	Total fungi
Harvest	78	18	10	41
Delayed harvest	34	31	49	88
Sign.[3]	**	*	**	**

[1] Means based on 4 seedlots of 50 seeds each at each harvest maturity. Assays based on potato dextrose agar plate assay at 27° C.

[2] Harvest at normal harvest maturity and 3 weeks later (delayed harvest).

[3] Statistical Significane (Sign.) based on FLSD; * = Stat. Diff. at P = 0.05, ** = Stat. Diff. at P = 0.01, and NS = no Stat. Diff. at P = 0.05.

was 2.8%, whereas, 62.0% recovery was found on pigeon pea tannin extract agar with surface disinfected seeds incubated at 35° C. Species of *Aspergillus flavus* group (11–56%) and *A. niger* group (3–19%) were predominant Aspergilli (table 7). Storage losses in seed viability measured by the difference between the cellulose pad germination values before and after storage for the same seedlots were not correlated with *Aspergillus* incidence on either of the cellulose pad assays ($r = 0.00$ NS and $r = 0.18$ NS, respectively). Nevertheless, when seeds were assayed selec-

TABLE 5.—*Effect of farmer storage (December to May) on pigeon pea seed viability and mycoflora in Puerto Rico*

Seedlot	Mean percentage[1]															
	Germination		*Botryo-diplodia*		*Fusarium*		*Phomopsis*		*Rhizopus*		*Penicillium*		*Aspergillus*		*Clado-sporium*	
	AH[2]	AP[2]	AH	AP	AH	AP	AH	AP	AH	AP	AH	AP	AH	AP	AH	AP
Coamo 1	65	61	18	5	16	1	6	0	0	2	0	6	0	3	4	5
Coamo 2	84	73	1	3	1	0	4	0	0	5	6	7	0	3	15	5
Villalba 1	73	51	3	0	4	1	0	0	1	6	0	5	0	3	24	5
Villabla 2	59	28	3	0	15	5	8	6	0	9	0	2	0	2	15	2
$\bar{x}$[3]	71	53	6	2	9	2	4	1	0	6	1	5	0	3	15	4
Sign.[4]	**		*		**		*		**		*		**		**	

[1] Based on 4-seed samples of 50 seeds each for each seedlot. Seeds were incubated on moist cellulose pads at 27° C.

[2] AH = at harvest maturity, i.e. before storage, and AP = at planting, i.e. after storage.

[3] Composite mean based on four seedlots for each storage treatment.

[4] Statistical Significance (Sign.) based on FLSD; 8 = Stat. Diff. at P = 0.05 and ** = Stat. Diff. at P = 0.01.

tively storage losses in seed viability were highly correlated with *Aspergillus* incidence ($r = 0.96^{**}$) (table 7). Table 8 shows all fungi which were identified from pigeon pea seed in these studies and their observed association with seed decay.

SOIL MOISTURE

Pigeonpea seeds did not emerge at either 25 or 100% MHC. Low emergence (6%) was found at 75% MHC and optimal emergence (36%) at 50% MHC. At 25 and 50% MHC *Aspergillus* species were the only fungi noted on and around seeds. At 75% MHC a mixture of *Aspergillus* species and pythiaceous fungi were present. Pythiaceous fungi were the dominant fungi at 75% MHC and the only fungi evident at 100% MHC.

TABLE 6.—*Comparison of the cellulose pad assay at 27° C to an* **Aspergillus** *selective assay (tannin rich pigeon pea seed agar at 35 C) for enumeration of* **Aspergillus** *species*

Seedlot	Mean *Aspergillus* (%)[1]		
	Cellulose Pad Assay[2]		Selective Assay[2]
	Before storage	At planting	At planting
Coamo 1	0	3	28
Coamo 2	0	3	60
Villalba 1	0	3	68
Villalba 2	0	2	92
$\bar{x}$	0	3	62
	FLSD between column means		P = 0.01 3.6

[1] Means based on 4 seed samples of 50 seeds each from each of the seedlots.

[2] In the nonselective assay seeds were incubated without surface treatment on cellulose pads at 27° C. In the selective assay seeds were surface-disinfected (4 min. 0.5% NaOCl) and then incubated on tannin rich pigeon pea seed extract agar at 35° C.

DISCUSSION

The results of these studies vary significantly from those of others looking at seed viability losses in pigeon peas. In these studies there were significant prestorage losses in seed viability in fields of the southern coast and foothills in Puerto Rico. Ellis et al. (3) found seed losses were restricted to the northern coast plantings, which are under more humid conditions. Agarawal (1) found that in India there was little evidence for sizable viability losses for pigeon peas either before or after normal ambient storage of 1 year. Difference between the Puerto Rico and India reports may be due to the fundamentally different seed types used in the different countries. Small hard seeds are used for split peas in India, while in Puerto Rico large vegetable types are preferred. In our studies, off-type 2B-Bushy pigeon pea with rather small hard seeds had greatly reduced invasion by seedborne fungi and greatly increased viability. In

soybeans, small hard seed lines are known to resist field and storage decay in comparison with large seeded lines (11, 12). Differences in the results of Ellis et al. (3) and ours may be related to changing cropping systems on the south coast. In our studies, pigeon peas had been continuously cropped for 3 to 5 years, whereas in the time of Ellis's research the same area was virtually virgin to pigeon pea, having been in a long-term sugarcane monoculture. Kmetz et al. (8) reported that seedrot from *Phomopsis* sp. in soybeans was greatly increased when fields were not rotated away from soybeans. Although Ellis et al. (3) suggested the south coast as an ideal seed producing area, it may not be ideal. The south coast of Puerto Rico has a constant high relative humidity and temperature which may not promote seed viability. Furthermore, although the

TABLE 7.—*Colonization of farm stored pigeon pea seed by various species of* **Aspergillus** *in Puerto Rico*

Seedlot	Mean Percentage (%)[1]			
	Aspergillus flavus	*A. niger*	Total *Aspergillus*[3]	Storage[3] germination loss
Coamo 1	14	3	28	6
Coamo 2	22	15	60	13
Villalba 1	11	19	68	30
Villalba 2	56	15	92	54

[1] Means based on four samples of 50 seeds for each seedlot. Seeds were plated on high tannin pigeon pea extract agar after surface disinfection of seeds (4 min. and 0.5% NaOCl). Incubation was at 35 C.

[2] Includes *A. tamarii*, *A. flavus*, and *A. parasiticus* (*A. flavus* group); *A. niger*, *A. pulverentus*, and *A. carbonius* (*A. niger* group); *A. glaucus* group; *A. restrictus* group; and unidentified species.

[3] Storage germination loss = germination value on cellulose pad at 27° C before storage (December) minus germination value using the same assay after storage (May).

total rainfall is low, occasional heavy rainfall and off-season rains are not uncommon. Desert areas of moderate temperature are the best areas for establishing seed production to avoid seed diseases.

In these studies the type of germination assay significantly affected the estimation of viability and seed mycoflora. Cellulose pad assay appeared to give near maximum germination values and to favor the detection of *Cladosporium* sp. and *Rhizopus* spp., probably because no surface disinfection was used. The agar plate assay allowed greater detection of *B. theobromae*, *Phomopsis* sp., and *Alternaria tenuissima*. These fungi may be more internally borne in the seed, may need greater external nutrients for their expression and may be sensitive to interference from *Cladosporium* and/or *Rhizopus* spp. Although superficial and internally seedborne fungi appear to interfere with each other in in vitro

assays, the relative sensitivities of these to soil fungistasis is not known, but is of practical importance.

Succession of seed mycoflora was first noted in delayed harvest and became increasingly evident after seed storage. *B. theobromae* appears as the most dominant fungus at normal harvest but is supplanted by *Fusarium* spp. after harvest delay. During storage all field fungi decline significantly but at varying rates. In these studies, *Fusarium* spp. declined more than *B. theobromae*, probably because more of their infections are

TABLE 8.—*Fungi recovered from pigeon pea seed and their frequency and association with preharvest and storage decay in Puerto Rico*

	Fungus	Frequency[1]	Decay
1.	*Alternaria tenuissima*	F	None
2.	*Arthobotrys* sp.	O	None
3–6.	*Aspergillus flavus* group *A. flavus, A. parasiticus, A. tamarii*	F	Storage
7.	*Aspergillus glaucus* group	F	Storage
8–10	*Aspergillus niger* group *A. carbonius, A. niger, A. pulverentus*	F	Storage
11.	*Aspergillus restrictus* group	F	Storage
12.	*Botryodiplodia theobromae*	F	Field
13.	*Colletotrichum gloeosporioides*	O	Field
14.	*Cladosporium* sp.	F	None
15.	*Fusarium sambucinum* var. *coeruleum*	F	Field
16.	*Fusarium semitectum*	F	Field
17.	*Macrophomina phaseolina*	O	Field
18.	*Monilia* sp.	R	None
19.	*Nigrospora sphaerica*	R	None
20.	*Penicillium* sp.	O	Storage
21.	*Phomopsis* sp.	O(F)	Field
22.	*Rhizopus* sp.	O	Storage
23.	*Trichothecium roseum*	R	None

[1] F = fungus recovered frequently, at times exceeding 20% in certain seedlots.
O = fungus recovered occasionally, recovery rate not exceeding 15% ever.
R = fungus rarely recovered and recovery rate never exceeding 5%.
(O)F = occasional in pigeon pea fields in semiarid areas, but frequent in humid zones.

superficial and because of the great resistance of the melanized hyphae of *B. theobromae* to adverse environmental conditions.

Ellis et al. (4) found no relation between *Aspergillus* spp. incidence and storage deterioration of pigeon peas. However, this is not due to absence of *Aspergillus* spp., but to the use of inappropriate isolation techniques. In Puerto Rico, where farmers are not adequately testing moisture of harvested seeds, storage fungi can be a significant problem. Potential production of aflatoxins or other mycotoxins in pigeon peas should be explored.

Farmers generally attribute pigeon pea stand losses to improper soil moisture when heavy rains follow planting. In these studies, a narrow range of soil moisture was favorable for optimal stands. Further studies should focus on how seed quality and seed treatment or other factors interact with the soil moisture level to determine stand density and vigor.

RESUMEN

En Puerto Rico se identificaron 23 especies de hongos en las semillas de gandul utilizando como substratos almohadillas de celulosa y medio de agar agar, papa y dextrosa (APD). *Botryodiplodia theobromae* fue el hongo más comúmmente encontrado en APD (29%). Comparándolo con las pruebas en celulosa, el APD hizo posible una alta detección de *B. theobromae*, *Alternaria tenuissima*, *Phomopsis* sp. y del total de hongos. En celulosa, se detectó una alta incidencia de *Cladosporium* sp. en semillas de gandul. En los dos tipos de ensayo se encontraron altas correlaciones negativas entre los valores de germinación del gandul y las incidencias de *B. theobromae*, *Fusarium* spp. y el total de hongos. Las medidas de viabilidad de la semilla en los dos ensayos resultaron altamente correlacionadas ($r = 0.77^{**}$).

Se estudió la influencia de la morfología de la semilla y de la cosecha tardía sobre la viabilidad de las semillas de gandul. Las semillas de la variedad 2B-Bushy, que eran grandes y de color crema, estaban más infectadas por hongos, especialmente por *B. theobromae* y *Fusarium* spp. También tenían menor viabilidad que las semillas pequeñas, duras y de color rojo oscuro de algunas líneas segregantes de la misma variedad. Una cosecha tardía de tres semanas aumentó drásticamente la incidencia de hongos y redujo grandemente la germinación de las semillas de gandul "kaki" en Santa Isabel.

Se analizó la viabilidad y la micoflora de las semillas de gandul en fincas pequeñas antes y después de almacenarlas. Después de almacenarlas la incidencia de *Fusarium* spp., *Phomopsis* sp., *Cladosporium* sp. y *B. theobromae* en las semillas eran 15, 26, 30 y 38% de la registrada antes de almacenarlas. Durante el almacenamiento aumentó la incidencia de las especies de *Penicillium*, *Rhizopus* y *Aspergillus*. En ensayos con celulosa como substrato a una temperatura de 27° C se notó una incidencia de 3% en los aspergilos. Sin embargo, a una temperatura de 35° C y en un substrato de 2% de agar agar con extracto de semilla de gandul, se encontró que la incidencia de *Aspergillus* era mayor (28 a 92%). Con este método más sensitivo para *Aspergillus* se encontró una alta correlación positiva ($r = 0.96^{**}$) entre la pérdida de viabilidad de la semilla después de almacenarse y la incidencia de *Aspergillus*. No se detectó ninguna correlación significativa entre la incidencia de *Aspergillus* en celulosa y la pérdida de la viabilidad de la semilla durante el almacenamiento ($r = 0.18$ NS).

El brote de la semilla de gandul y su ataque por hongos dependió de la saturación del suelo con agua. A 25 y 100% de saturación las semillas no brotaron. El mejor brote ocurrió cuando había 50% de saturación. Los ficomicetos, aparentemente *Pythium* u otros hongos similares, colonizaron la semilla de gandul predominantemente a una saturación de 75% o más. Las especies de *Aspergillus* predominaron en las semillas a una saturación de 50% o menos. Se observó una densidad mixta de los diversos grupos de hongos a 50 y 75% de saturación.

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